

2003 *Ralstonia solanacearum* race 3 biovar 2

- Outbreak in Geraniums: A Program Review -

**- Detections on Solanaceous Crops in the US -
Meeting Summary**

Introduction

Two meetings dealing with *Ralstonia solanacearum* race 3 biovar 2 were held at USDA Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine headquarters in Riverdale, MD from June 17 to 19, 2003. The first was a one and one-half day program review to look at the Plant Protection and Quarantine (PPQ) response to the 2003 detection and control program for *Ralstonia solanacearum* race 3 biovar 2 (Rs r3b2) in geraniums. The second meeting was to gather up-to-date information on the detection and control of Rs r3b2 in solanaceous crops, including potatoes and tomatoes in the U.S.

The program review was conducted with reports and case studies from various program managers, USDA scientists, State Plant Health Directors, and PPQ Officers, with panel discussions and participation from various USDA programs, the National Plant Board, scientists from states and university diagnostic centers, and geranium industry representatives.

During the second meeting, following a morning of presentations on the current body of the scientific and operational knowledge, the afternoon was opened up for a wide-ranging discussion of the necessary elements of a PPQ action plan for field crops. Several state seed potato certification scientists and a representative from the vegetable industry provided important input into the PPQ planning process.

It was generally agreed that the time and place to deal with Rs r3b2 is prior to importation of potential host material into the United States by the application of appropriate sanitary controls and practices at off-shore production facilities. The 70 attendees at both meetings benefited from input and presentations given by distinguished scientists, recognized as the world's leading experts in the spread, diagnosis and control of Rs r3b2. What follows is a summary of key points discussed during the two meetings.

Key Discussion Points

Biology and Epidemiology:

- Environmental Requirements
 - Temperature: biovar 2 is thought to be more cold tolerant than the

- others.
 - Holding plants at higher temperatures does not necessarily lead to symptom development
 - Humidity: May play a larger role than originally perceived, but not well characterized.
 - Survives in plants for up to 10 years.
 - Best in the early spring when there are warm moist conditions.
 - Best following a flood or over-irrigation.
- Infection:
 - Is infection due more to natural processes or wounding?
 - Data is inconclusive.
 - Confirmed that it is not clonal, i.e. plant parts breaking off and becoming re-established further down stream
- Hosts
 - The only weed host perceived to be significant is *Solanum dulcamara* (bittersweet or climbing nightshade).
 - In Europe, where established, the roots spread into the water and become infected.
 - This weed is generally a latent host (asymptomatic)
 - Variability when symptoms are present. May include:
 - Brown staining of vascular tissue
 - Some Wilting
 - Some Soft Rot.
 - Wild indigenous species in U.K.
 - In the U.S. *S. dulcamara* is an introduced weed and is now widespread.
 - Neither *S. dulcamara*, nor the bacteria die in freezing temperatures.
 - Present in *S. nigrum* (black nightshade) much less frequently
- Environmental Spread:
 - Initial infection is a rare event, but once established, spread is quite rapid.
 - Long-term survival occurs in water, soil, or latent hosts.
 - Ground/River Water:
 - Most important means of dissemination in Europe.
 - Irrigation water was identified as a pathway
 - Strong relationship between *S. dulcamara* and irrigation water.
 - Can always be traced back to a water plant.
 - Numbers drop below detectable levels in winter (not multiplying).
 - Inoculum present in rivers is quite high.
 - Can be detected in host plant in the winter.
 - Original source of European infection may have been imported potatoes from Egypt contaminating commercial potato waste or domestic waste stream
 - Sewage Effluent
 - Always traced back to a sewage outfall.

- Probably an historical introduction or crude waste is bypassing treatment.
 - The organism is not currently entering the waterways with any frequency.
 - Negligible risk of survival after sludge treatment.
- Greenhouse Spread:
 - Anecdotal evidence suggests multiple introduction points, or disease spread (No experimental evidence).
 - No plant-to-plant spread was observed in Kenya in standing water.
 - Disease incidence is variable greenhouse to greenhouse and year to year.
 - Rooting stations are a crucial step in spread (Industry is developing BMPs to address these risks).
- Viable but not Culturable (VBNC) organisms — Organisms that can reproduce and infect a host plant, but which can not be cultured on selective media.
 - Scientific community disagrees about the nature of these organisms.
 - Increased copper and decreased temperatures are thought to contribute to this condition.
- Competition with other organisms:
 - Race 1 inhibits race 3 under optimal overlapping conditions.
 - Environmental (Temperature) conditions may change the response in the field.

Survey and Detection:

- Latency, infected but asymptomatic plants, is a major challenge in the greenhouse and the environment
- An environmental survey has been discussed.
- How much of potato crop would be tested, and what are the associated costs?
 - Unsure of exact figure in the U.K., but it is listed in the control directive. In addition, member states may increase the levels of testing beyond those specified.
- Short-term Goal: Educate people who may encounter infected commodities.
- Informal waterway checks in table and seed potato areas via PCR and sentinel tests have been discussed.
- A long term survey has been discussed
- Agdia, Inc. is developing special water testing kits.
- It was suggested that ditch irrigation systems will be more amenable to

finding organisms in the Pacific Northwest.

- How survey and detection work in areas of the U.S. that already have race 1 biovar 1 needs to be worked out.

Control of Rs infection

- Sanitation products — Effective products available but not labeled appropriately
 - Bleach powder has been used.
 - Quaternary ammonium compounds and hydrogen dioxide used in greenhouses
 - Methyl Bromide is only 60% effective.
 - Chloropicrin is more effective, but not 100%.
 - Thymol might be effective.
- Hygienic Protocol — Better management practice than relying on sampling
 - Entry/exit sanitation
 - Compartmentalize irrigation within greenhouses to minimize issues such as backflow and seeping of infested water.
 - Separation of product
 - Disinfection between pinching and cutting.
- Culture indexing of “mother” plants
- Steam Sterilization:
 - Must be deep and thorough
 - U.S.D.A. treatment manual requires steam plus pressure (autoclaving)
 - Composting temperatures generally kill all of the pathogen.
- Eradication:
 - Spraying *S. dulcamara* with herbicides has caused reductions of the weed host in many areas.
- Breeding for resistance
 - Resistance may allow for latent infection in vegetatively propagated plant
 - Need for clean seed/ cutting program for vegetatively propagated host crops
- Field infection — ban on (potato) production for several years
- Proper disposal measures, including permitted land fills
- Shared machinery restrictions
- Store contaminated water over the winter in storage lagoons which are free of host plants. These lagoons will usually test negative in the spring.

- Cover crops: If the planted crop does not support populations, then you will not have weeds supporting populations.
- Fallowing is largely ineffective.

Laboratory Diagnostic Techniques:

- Immunofluorescence (IF):
 - Required by European law as a first screening test.
 - Antibodies specific for species, **NOT** race or biovar
 - Sensitivity — 10^3 to 10^4 cells per ml
 - Gives some idea of quantity and allows for direct visualization of the bacterial cells themselves.
 - Technique already used in the US for seed potato certification for ring rot.
- Pure Culture Isolation on Selective Media:
 - SMSA — selective media with several recipes
 - Elphinstone has recipe that is not toxic to variant strains
 - Source of reagents is important
 - Time Requirements — 2 days for initials, 5 days for colony morphology
 - Required by European law as second step in screening process (in response to an IF positive).
 - Not done in recent US event due to lack of manpower and facility space
 - Can be used in bulk sampling (useful in testing asymptomatic plants or at ports of entry)
 - It is easier to do PCR with cultured organisms, and the culture allows you to maintain a legally valid record
 - Can be used for sampling water
 - Can reach biovar by using a series of differential select media
 - VBNC organisms require different culturing techniques
- Pathogenicity Testing:
 - Required by European law as third step in screening process (in response to IF positive and pure culture isolation)
 - Tomato is used as host
 - For the recent event, some colonies were isolated and inoculated on geranium — 100% infection rate after 3 days on 2 cultivars.
- Polymerase Chain Reaction (PCR):
 - More rapid than other screening tests
 - Concerns:
 - Degree of sensitivity among sequences targeted by I.M. Lee primers.
 - Multiple PCR tests:
 - CSL Multiplex with internal control that checks for false negative reactions.
 - CSL TaqMan Quantitative Real-Time PCR

- Quantitative
 - Growth during enrichment phase indicates pathogen viability
 - Compatible with Cepheid Smart Cycler platform
 - Dye incompatibility with newer equipment at NPGBL prevented use of this protocol during 2003 introduction
- ARS Test (developed by N. Schaad of ARS): essentially a modified version of CSL, still under development, not available, still being modified.
- ARS (I.M. Lee)
 - primers target insertion sequence, multiple copies
 - PCR was immediately available, so it was used
- Enzyme Linked Immunosorbent Assay (ELISA):
 - Needs very high cell numbers to work in most cases.
 - Current antibodies only specific to species, cannot determine for race or biovar. Positive result is an indicator for further testing.
 - Types:
 - CSL (ELISA with a highly specific Monoclonal antibody): Good level of sensitivity, extensively checked for cross-reactions, good for field testing in symptomatic plants, and also testing for *Xanthomonas* available in the same format.
 - Agdia — Agden Spot Check: positives sometimes less distinct, less tolerant of extended incubation
- Symptomatology: varied between sites, often subtle and easily missed, included upward reaching leaves, gray leaf syndrome, chlorotic to necrotic wedges, eventual and wilt
- Water Streaming Tests
 - Oozing is not always diagnostic: Presence of streaming suggests *Xanthomonas* or *Ralstonia*, but if it is missing, you can not confirm the bacteria are absent (may still be a latent host).
- Sample Handling and Submission:
 - Need consistent, well-defined methodologies
 - Best samples come from the entire section of stem (including **ALL** xylem tissue) at the base of the plant.
 - Suggestions to test water coming out of pots as a screening technique
 - PCR and ELISA would not necessarily work in this situation.
 - Culture on SMSA would be the best bet
 - There is potential to test via water filters at rooting stations
- Ring Tests — European method for validation of diagnostic testing protocols, guidance lab with several other testing labs. New technology is simultaneously tested in many places to ensure that the methodologies are robust and work in many places.

Operational Issues:

- What is a legal positive?...What is a legal negative?
 - It is important not to rely on any one given test. All tests will have pros and cons.
 - Depends on legal status. In the UK, a culture is required before a positive confirmation. Would the U.S. take action on grounds of suspicion, or would the U.S. require proof — Koch's Postulates (6 weeks to 2 months).
 - Would the U.S. operate on two levels: suspect and positive?
 - Take holding action before confirmation.
- What if a positive sample is detected off-shore?
 - Entire facility would be held
 - Trace Back/Forward (Industry is voluntarily creating barcode tagging to help)
- The only greenhouse model that was available to work with for the development of APHIS *Ralstonia* Emergency Action Plan was Chrysanthemum White Rust.
- Select Agent Status:
 - Creates a significant administrative burden for diagnostic labs: permitting, record keeping, etc.
 - Creates confusion regarding access of non-viable forms, genetic elements, etc.
 - Exemptions are being developed.
 - Mandated review of list, with expert consultation required.
- Because of Ag-bio-terrorism rule, there were some kinks that slowed the processes, i.e. permitting, approved structures, etc.
- Legal mandate that a federal lab must confirm “first finds.”
- Action Plan:
 - Pros:
 - Prepared in two weeks
 - Was made available on the APHIS Web site.
 - Some states already had large networks of inspectors.
 - Cons:
 - Need clearer definitions of:
 - “Commingling”
 - “Disposal and Destruction”
 - “Shipment Destruction vs. Rouging”
 - “Latent Infection”
 - Time/Temp tables need refinement.
 - Not necessarily amenable to all levels of variability within the industry.
 - Information exchange needs:
 - Smoother integration from the field to the office.
 - Timely response to sample submission, and clear definitions of who will receive the response.

- Inspector education (Infected plant ID and complete knowledge of action plan)
 - Honesty about compensation
 - Public education (early detection, hygienic practices, definitions of hazardous waste/select agents)
 - Cooperation between federal and state inspectors.
 - Changing plans/Updating Drafts created confusion
- Phytosanitary Pre-Certification — A series of inspections based on Best Management Practices (BMP) document
 - The protocol that is created should be open to new methods for survey, detection, and control.
 - Aimed at eliminating future introductions of Rs via geranium pathway

Geranium Grower Issues:

- Growers often would prefer to simply destroy plants as soon as Ralstonia is detected. To them, race and biovar is just an issue of quarantine. They don't want any race/species in their greenhouses.
- Find it hard to justify destruction of their crops for the protection of the potato and tomato industries, because Ralstonia is not a huge threat to the geranium industry.
- Will grower practices, such as copper compound application, or chilling of samples affect testing issues, namely VBNC?
 - These two factors could lead to VBNC. They will not affect enzyme linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR). Generally, to induce VBNC, the organism should be chilled to 4oC in soil or canal water for longer than a week. Pure culture isolation on SMSA may be affected.
- Timely response to sample submission, and clear definitions of who will receive the test results.
- Had trouble finding approved and affordable landfills in reasonable proximity to the affected greenhouse that would accept the waste. Incineration and autoclaving were also limited by cost.
- Are growers who are prohibited from growing compensated in the Netherlands?
 - In the beginning growers were given 50% by the government and 50% by the industry.
 - At this point in time an insurance system has been set up by the industry

Industry Issues (Geraniums):

- Does not allow wholesalers who purchase cuttings and root them to sell to other growers.
- Does quarantine mean that everything (or only geraniums) is prevented from moving from the greenhouse?
 - Initially, all movement is place on hold. The inspector verifies the location of suspect plants, and the rest of the greenhouse is released. The Time/Temp Table will still be used, and continued improvement will occur.
 - In case of special circumstances, holds will be placed accordingly.
- Are there any studies that confirm or suggest that the organism can move from a geranium to a potato?
 - This would most likely be dependant on the pathogens movement into a watercourse.
- The industry could not abide by a 2 month lag time required for Koch's Postulates
- Pure Culture Isolation

Industry Issues (Potato):

- Any detection procedures should be aimed at detecting it not only in the field, but also during shipment.
- Within the nationwide seed potato certification program, the short term goal should be to educate people. There is currently no knowledge of this organism in the potato industry.
- There is a need to monitor the environment. Once you see the wilt, then it is too late.

Research Questions:

- Should *Ralstonia solanacearum* be divided into more than one species?
- How common is latency in geraniums and other ornamentals?
- How easily does the pathogen spread from geranium to geranium?
- What is the relationship between temperature and disease development in geranium? Effects of humidity?
- More information about the spread and epidemiology in greenhouses is needed.
- What is the main mode of infection (wounding vs. natural) in both greenhouse

and field?

Disclaimer: This summary is not based on a verbatim transcript of the meeting session and may not be a complete and accurate record of the presentation and discussion. It is provided only as service to meeting attendees and other interested parties. Any questions or comments concerning the meeting should be submitted to [Jim Writer](#), USDA APHIS PPQ.